conceivably function as a component of a primary signal for new RNA and protein biosynthesis. More likely the primary effect of the cholinergic stimulus is to increase the secretory rate of the gland. RNA and protein synthesis may then be stimulated by a signal that is generated by the release of stored protein. Experiments with the ampullate silk gland of the spider have shown that acetylcholine causes the secretion of preformed silk protein followed by increased protein synthesis(9). In this case puromycin inhibits the increased synthesis of new protein but does not affect the initial secretion.

Summary. It is found that the rate of synthesis of RNA is increased in the pigeon pancreas following cholinergic stimulation. This increase occurs in the first hour following stimulation. There is also an increase in the rate of protein synthesis following cholinergic stimulation. Both of these results, while in agreement with current theories of protein synthesis, are in disagreement with previously published work on the cholinergic stimulation of the pigeon pancreas.

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Studies of the Diabetogenic Action of Streptozotocin.* (32401)

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Streptozotocin is an antibiotic extracted from *Streptomyces acromogenes* and prepared in highly purified form. Its molecular weight is 265 with the empirical formula $C_8H_{15}N_3O_7$ containing a N-nitrosomethylamide function(1). The substance has also been shown to exert antitumoral activity in leukemia L 5178 Y, Ehrlich carcinoma and Walker 256 carcinosarcoma(2). In 1963, Rakieten *et al* (3) further reported that streptozotocin is diabetogenic, since its intravenous administration led to frank diabetes in dogs and rats. On the basis of their histologic studies, they attributed this diabetes to damage to the pancreatic B-cells. However, Evans *et al*(2), while confirming the diabetogenic action, suggested that it might not result from permanent damage or necrosis of the B-cells, but rather from an inhibition of production and/or secretion of insulin. Similarly, Arison *et al* (4) have concluded from their studies, including electron microscopy, that streptozotocin produces degranulation of B-cells without necrosis.

In all of the studies reported so far, some doubt remained as to the purity of the streptozotocin preparation, since Evans *et al*(2) stated that many preparations made available prior to 1965 were contaminated with as much as 15% of another compound, Zedalan (3-[oximinoacetamido]acrylamide, U-15,774). Indeed, these authors suggested

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that the combined action of streptozotocin and Zedalan might be required for both antitumoral and diabetogenic activity.

During the last 18 months, we have undertaken a systematic study of the diabetogenic action of streptozotocin. While some of the preparations first used contained the contaminant Zedalan, only preparations conforming to strict standards of purity have been employed during the last year and have been made generously available to us through Dr. C. G. Smith of the Upjohn Research Laboratories, Kalamazoo, Mich. These studies lead us to conclude that pure streptozotocin has a specific, rapid and irreversible cytotoxic action upon pancreatic B-cells, which only partly resembles that of alloxan.

Materials and methods. Only results obtained with pure streptozotocin will be reported here. The substance was dissolved in saline to which a few drops of citric acid 0.05 M were *promptly* added, pH being adjusted to 4.5 in less than one minute. This is a necessary precaution in order to prevent the rapid inactivation of streptozotocin at neutral pH. As a rule, the concentration of streptozotocin in the solution for injection was 10 to 30 mg per ml. It was injected into the saphenous vein of male rats of Wistar strain, bred in our colony, and weighing between 165 and 230 g. The animals were fasted for 16 hours prior to the injection, which was carried out under light ether narcosis. After the injection, the animals which were to be killed after 1, 2, 4, 7 and 10 hours remained fasting until that time, while the others were allowed access to food up to 16 hours prior to death by decapitation, under anaesthesia with nembutal i.p. Blood serum was used for measurements of glucose (5) and of immunoreactive insulin (IRI) according to a double antibody technique slightly modified from those of Hales and Randle(6) and Morgan and Lazarow(7). Two-thirds of the pancreas, dissected free of fat, was immediately frozen and later extracted with acid-ethanol(8) with subsequent measurement of pancreatic IRI by the same immunological technique. The remaining third was fixed in Bouin for histological examination. The staining techniques used were hematoxylin and eosin, aldehyde-thionine according to a slight modification of Paget's procedure(9), chromium-hematoxylin-phloxin (10) and impregnation with silver according to Hellman(11). For electron microscopy, some samples were fixed by perfusion with a 2.3% solution of glutaraldehyde in cacodylate buffer, the osmolarity being adjusted to 300 mosm, and postfixed in a 1% solution of osmium buffered with s-collidine(12); the other samples were fixed by immersion in a 2.3% solution of glutaraldehyde buffered with cacodylate or phosphate and postfixed as described above.

Throughout the experiments, the animals were kept in metabolic cages, and, with the exception of those killed within 10 hours of the streptozotocin injection, they were allowed pelleted rat laboratory chow *ad libitum*. Weight, urine volume, urine glucose and ketone bodies were recorded throughout.

Results: The data obtained in a group of 60 animals given a single intravenous dose of 65 mg streptozotocin per kg are summarized in Fig. 1 and 2. There was an early

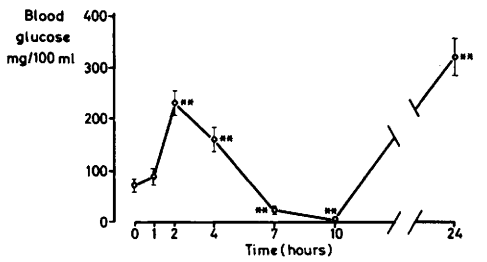

FIG. 1. Effect of intravenous injection of 65 mg/kg streptozotocin on serum glucose levels in rats (5 to 7 for each point) fasted 16 hrs (mean values ± SEM).

increase in blood glucose levels at 2 hours with a marked subsequent decrease, hypoglycemia being most pronounced in the samples obtained at 10 hours. This hypoglycemic period was followed by hyperglycemia which then became permanent. All blood glucose values shown are values obtained after a fasting period of at least 16 hours. Glycosuria began during the first day and increased in magnitude during the first week, with parallel increases in urinary volumes to between 100 and 150 ml per 24 hours, as compared with

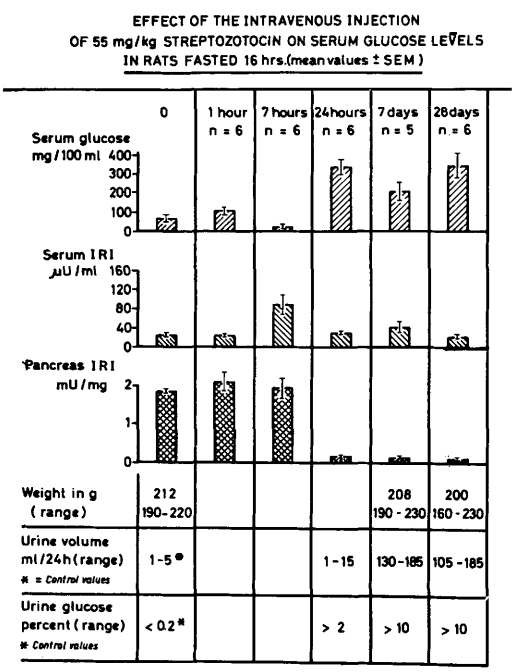

FIG. 2. Effects of intravenous injection of 65 mg/kg streptozotocin in rats (5 to 7 for each group) fasted 16 hrs (mean values ± SEM).

control values not exceeding 1-5 ml per 24 hours. Ketonuria was rare, and an early decrease in urine volume was not seen. Body weight remained, on the average, at or near the initial value, while control rats at the same stage of development gained approximately 30-40 g weekly. Despite the absence of ketonuria, lipemia may be pronounced, both quite early (after 2 days) and quite late (up to several months) after the streptozotocin injection. Decrease in abdominal depot fat was definite after one week, while fat stores had practically disappeared by one month.

As also shown in Fig. 2, there was a highly significant increase in serum IRI after 7 hours, coinciding with hypoglycemia. Serum IRI then returned to fasting levels despite the pronounced hyperglycemia. Pancreatic insulin content remained normal up to 7 hours, then decreased to levels persistently below 5% of normal after 24 hours and 2% of normal after one month.

At this dose level, there were no deaths other than those attributable to hypoglycemia at approximately 10 to 15 hours. Such deaths can easily be prevented by allowing the animals access to food soon after completion of the injection.

Histology. The main findings concerned the endocrine pancreas. After one hour, some widening of the pericapillary spaces may be seen in the islets of Langerhans, as well as occasional pyknosis of the nuclei of the B-cells, as yet without degranulation (Fig. 3). After 7 hours, massive necrosis of the B-cells was evident, with cellular disintegration and often nearly complete caryolysis (Fig. 4). Both then and later, the remaining B-cells were rare and mostly degranulated. The A-cells and the exocrine pancreatic tissue remained intact and without any evidence of damage throughout.

The anomalies observed in kidney and liver were those expected in the presence of hyperglycemia and hyperlipemia. There was some evidence of focal hepatic necrosis after 2 to 36 hours, but no other evidence of gross direct toxic damage of streptozotocin in liver, kidney and other tissues. There was some early hyperplasia of hepatic Küpffer cells.

Electron microscopy. Observations with the electron microscope confirmed and established the early occurrence of necrosis of the B-cells with vesiculation and complete disruption of cytoplasmic structures, despite an entirely normal appearance of the cytoplasm of A-cells seen in the same field (Fig. 5). As early as 6 hours after administration of streptozotocin, there was also clear-cut evidence for the presence of macrophages actively phagocyting B-cell cytoplasm and granules (Fig. 6). It is of interest to note in both the electron micrographs shown that the membranes surrounding the β granules appear to be more resistant during generalized cytoplasmic necrosis than either the plasma membrane or the membranes of the endoplasmic reticulum.

Discussion. In contrast with previously expressed reservations, our studies unequivocally establish that pure streptozotocin is a remarkably effective cytotoxic agent for pancreatic B-cells. The action occurs early, since histologic evidence can be obtained after 1 hour, with intense necrosis strictly limited to B-cells evident at 7 hours. This necrosis is

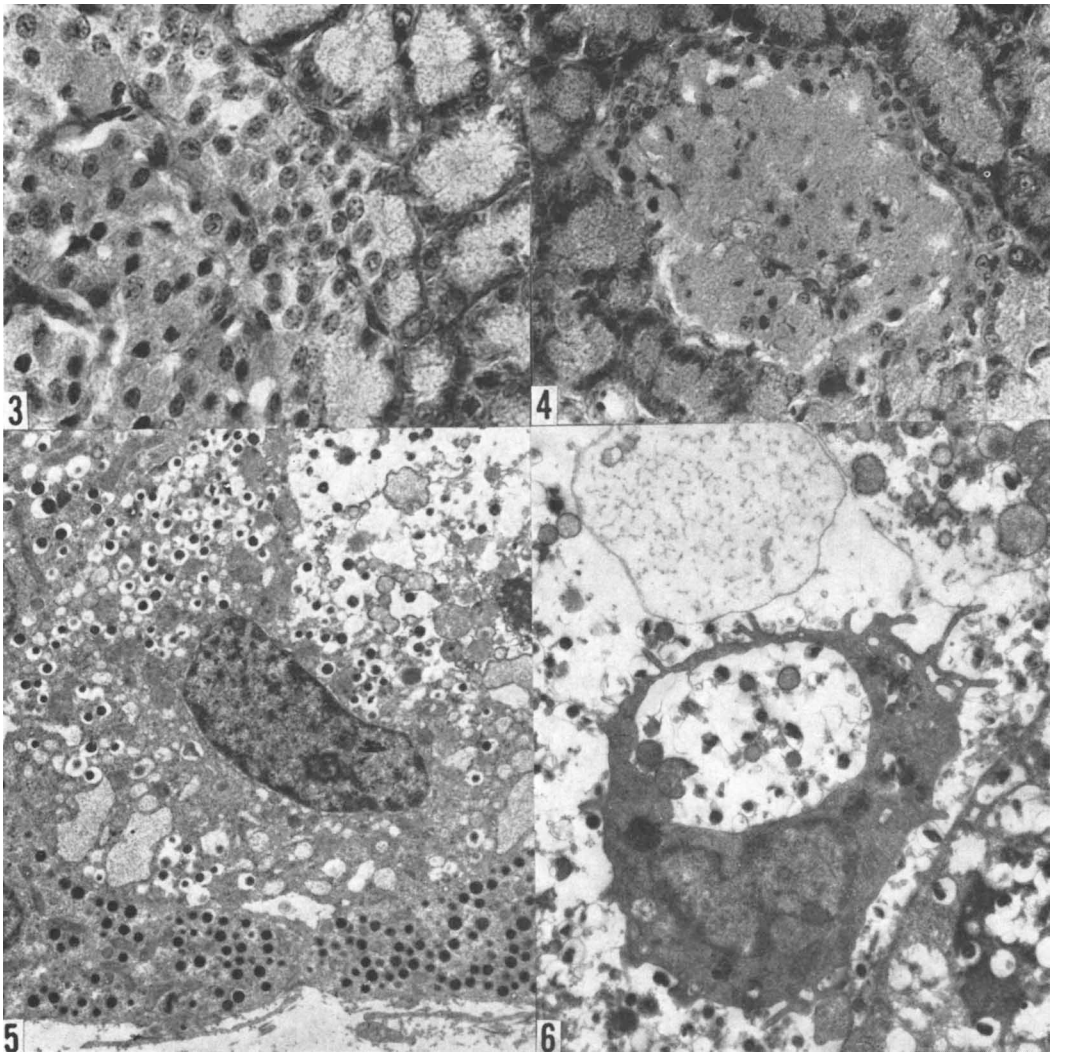

FIG. 3. Islet of Langerhans, 1 hr after injection of 65 mg/kg streptozotocin. Note the pyknosis affecting several nuclei of B-cells. Aldehyde-thionin, × 590.

FIG. 4. Islet of Langerhans, 7 hrs after administration of 65 mg/kg streptozotocin. Nearly complete necrosis of B-cells, while A-cells and exocrine pancreas are undamaged. Hematoxylin and Eosin, × 400.

FIG. 5. Electron micrograph of islet of Langerhans, 6 hrs after administration of streptozotocin. Below, 2 intact peripheral A-cells. In the center, one B-cell showing the first stage of damage: vesiculation of the granular endoplasmic reticulum. Above, on right, a necrotized B-cell and, on left, a still normal portion of another B-cell, × 3200.

FIG. 6. Electron micrograph of islet of Langerhans, 6 hrs after administration of streptozotocin. Area of necrosis. Note preservation of the membranes of the vesicles surrounding B-granules. In the center, one macrophage containing cellular debris at different stages of digestion, × 9600.

associated with release of part of the insulin contained in the necrotizing cells, as evidenced by the elevation of serum insulin levels. The best and most striking evidence for B-cell necrosis is obtained early, within 24 hours of administration of the drug, and this may be why previous authors have questioned its occurrence. Several histologic features, as well as the triphasic blood glucose sequence, suggest a strong resemblance between the B-cytotoxic action of streptozotocin and that of alloxan. However, several

observations also suggest differences between streptozotocin and alloxan action, with a considerably greater specificity of the streptozotocin effect upon B-cells than is true for alloxan. Thus, the general toxicity of the drug is very much less, particularly as far as nephrotoxicity is concerned. This greater specificity is best seen in the fact that clear-cut diabetes, although of lesser severity, has been observed with doses as low as 25 mg per kg, and diabetes of greater severity with doses as high as 100 mg per kg in the rat, while the LD_{50} in the rat has been reported to be about 130 mg per kg(3). This is in striking contrast with the action of alloxan which, in almost all species where it is effective, exhibits an extremely narrow margin between the diabetogenic and the generally toxic and lethal doses.

While much remains to be done in order to obtain a complete description of the pharmacology of the B-cytotoxic action of streptozotocin in several species, and while it is too early even to speculate as to the possible mechanism of action of the drug, it would seem clear that streptozotocin provides a new tool for production of experimental diabetes in a more graded and specific fashion than is true for alloxan. It would seem reasonable to expect that the drug may be of use in certain instances of malignant insulin-producing tumors. Finally, it is difficult not to be intrigued by the combination, in a single substance of rather low molecular weight, of 3 major biologic activities: antibiotic, anti-tumoral and B-cytotoxic.

Summary. Streptozotocin is a highly effective cytotoxic agent for pancreatic B-cells. After intravenous administration of 65 mg streptozotocin per kg, damage to B-cells is apparent as early as one hour after intravenous administration of the drug. Frank necrosis associated with phagocytosis is best seen after 7 hours, when pancreatic insulin release and hypoglycemia are also noted. By 24 hours, pancreatic insulin content is reduced to 5% of normal or less. While the B-cytotoxic effects of streptozotocin resemble those of alloxan, their specificity is very much greater, as demonstrated by the wide margin between diabetogenic dose and general toxicity.

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Studies on the Antipyretic Action of Salicylates.* (32402)

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Recent work on the pathogenesis of fever suggests that most fevers are caused by the action on the central nervous system, probably the hypothalamus, of an endogenous pyrogen released from leucocytes, as shown in Fig. 1(1-4). Similarly, defervescence of fever after treatment with salicylates has generally been attributed to their direct action on the hypothalamus, with resulting peripheral vasodilation and heat loss(5-8). Al-

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